

# Axial regionalization in *Tiktaalik roseae* and the origin of quadrupedal locomotion

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## Summary:

The origin of quadrupedal locomotion in tetrapods entailed the evolution of a regionalized axial skeleton with sacral ribs. These ribs provide linkage between the pelvis and vertebral column and contribute to body support and propulsion by the hind limb. The closest relatives of limbed vertebrates are not known to possess such a connection and, therefore, have typically been described as primarily supporting their bodies against the substrate with pectoral fins. However, data on the axial skeletons of stem tetrapods are sparse, with key features of specimens potentially concealed by matrix. Here we provide micro-computed tomography data of the axial skeleton of *Tiktaalik roseae* and show that its vertebrae and ribs are regionalized along the craniocaudal axis, including expanded and ventrally curved ribs in the sacral region. The sacral ribs would have contacted the expanded iliac blade of the pelvis in a soft tissue connection. No atlas-axis complex is observed, however the basioccipital-exoccipital complex is deconsolidated from the rest of the neurocranium, suggesting increased mobility at occipital-vertebral junction. Thus, axial regionalization that allowed for innovations in head mobility, body support and buttressing the pelvic fin evolved prior to the origin of limbs.

31 The earliest limbed vertebrates are characterized by a regionalized axial skeleton with cervical,  
32 thoracic, sacral, and caudal domains in the vertebral column and ribs<sup>1-4</sup>. These modules  
33 correspond to locomotor specializations, including providing support for load-bearing hind  
34 limbs<sup>5-7</sup>. *Acanthostega* and *Ichthyostega* have specialized ribs that connected to the ilium, either  
35 in a soft-tissue or bony articulation, providing mechanical linkage between the axial column and  
36 the pelvic girdle<sup>1,2,4</sup>. The structures that make this connection possible are not present in  
37 tetrapodomorph outgroups where the complete axial column has been described<sup>8-10</sup>. For example,  
38 the tristichopterid *Eusthenopteron* has vertebrae and ribs that are short and generally similar  
39 across their cranio-caudal distribution, lacks a sacral rib, and has a pelvis that is small as  
40 compared to the pectoral girdle<sup>8</sup>. Moreover, unlike *Acanthostega* and *Ichthyostega*,  
41 *Eusthenopteron* possessed a bony linkage between the shoulder girdle and cranium that would  
42 have limited head mobility<sup>1,2,4,8</sup>.

43 Little is known of the axial columns of the closest relatives of limbed vertebrates. The vertebrae  
44 of *Panderichthys* are described from a brief series that evince no indication of regionalization<sup>11</sup>.  
45 The vertebrae of *Elpistostege* are known from a series of approximately 16 that, likewise, show  
46 no heterogeneity in their length or shape<sup>12</sup>. The axial skeleton of *Tiktaalik* has been largely  
47 obscured by matrix. The rostral ribs are broad and laterally expanded as compared to early  
48 tetrapodomorph conditions, and vertebral column has not been described<sup>13</sup>. However, the pelvis  
49 and pelvic fin of *Tiktaalik* are nearly the size of the pectoral appendage<sup>14</sup>, differentiating its  
50 overall proportions from less crownward taxa, like *Panderichthys*<sup>11,15</sup>. The size and depth of the  
51 acetabulum, the general robusticity of the pubis, and the dorsally expanded iliac blades of  
52 *Tiktaalik* are further similarities shared with digitated forms that are notably absent in other finned  
53 tetrapodomorphs<sup>14</sup>.

54 Here, we present high-resolution micro-computed tomography scans of the type specimen of  
55 *Tiktaalik*, NUFV 108, that expose for the first time the vertebral skeleton and posterior ribs of  
56 *Tiktaalik* (Fig. 1, Movies S1,2). These data, and the new reconstruction they allow, reveal  
57 unexpected intermediate conditions as well as apomorphies that provide new insight on changes  
58 involved with the origin of limbed vertebrates and the functional context in which they arose.

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## 60 **Results**

### 61 *Vertebrae*

62 The vertebrae of *Tiktaalik* are rhachitomous and surround an unconstricted notochord that was  
63 persistent into adulthood (Fig. 2). In specimen NUFV 108, elements of 40 vertebrae are  
64 preserved. These include ossified intercentra and neural arches, while pleurocentra are not  
65 identified. The size, shape, and spacing of intercentra and neural arches of *Tiktaalik* are similar to  
66 *Eusthenopteron*<sup>1</sup>, suggesting that pleurocentra might have been present but, because of their  
67 small size, are difficult to identify within the field of preserved scales. However, their absence in  
68 *Tiktaalik* cannot be excluded; pleurocentra have likewise not been identified in *Panderichthys*<sup>11</sup>  
69 or *Elpistostege*<sup>12</sup>, suggesting a possible ‘reverse’ rhachitomous pattern in elpistostegalians which  
70 is known in *Acanthostega*, *Ichthyostega*, and *Pederpes*<sup>3</sup>.

71 Vertebrae are not preserved in association with the four most anterior ribs (Fig. 2 A,B). It is  
72 possible that these vertebrae were present but are not preserved in specimen NUFV 108.  
73 However, a similar condition is observed *Ichthyostega*, where preserved intercentra and neural  
74 arches also initiate at rib number five<sup>3</sup>. This well-defined gap in multiple taxa suggests that these  
75 vertebrae in the cervical domain were cartilaginous into adult stages in *Tiktaalik* and  
76 *Ichthyostega* and that the observed pattern is not an artifact of preservation or variation across  
77 ontogeny.

78 Intercentra are paired and have minor graded differences in their morphology across the series  
79 (Fig. 2 C). Proceeding caudally, intercentra become longer in the rostro-caudal direction, shorter  
80 dorsoventrally, and bear a larger articular facet for the ribs (Fig. 2 D-G). Similar rostro-caudal  
81 variation is observed in the presacral intercentra of *Eusthenopteron*<sup>8</sup>. *Tiktaalik* is distinguished  
82 from closely related taxa in having paired intercentra along the full series. In *Eusthenopteron*, the  
83 anterior five intercentra and the intercentra above the pelvis, at approximately position 32, are  
84 bilaterally fused<sup>8</sup>; *Acanthostega* has fused atlantal and sacral intercentra<sup>1</sup>; and in *Ichthyostega*  
85 most intercentra are fused, with only the anterior-most ones being paired<sup>3</sup>.

86 Neural arches are inclined posteriorly and vary craniocaudally in their morphology. Frequently,  
87 they are laterally compressed in preservation, with the left and right halves occasionally

88 separating, as has been described in *Eusthenopteron*<sup>8</sup>, *Panderichthys*<sup>11</sup>, *Elpistostege*<sup>12</sup>, and  
89 *Acanthostega*<sup>1</sup>. Zygapophyses are not observed, unlike the condition of limbed vertebrates<sup>2,3,16,17</sup>.  
90 Cranially, neural arches have a simple saddle shape (Fig. 2 D,E). The rostral 30 arches show  
91 subtle variation in their geometry, with more caudal neural arches having slightly more vertical  
92 inclination relative to the notochord. By position 32, the neural arch pattern shifts abruptly, and  
93 neural arches extend further dorsally and have a dorsal foramen. Neural arch 31 is broken  
94 dorsally, and so it is unclear whether the transition in neural arch morphology occurs at position  
95 31 or 32. Regardless, this shift in morphology is inferred to mark the trunk-tail boundary, a  
96 change also observed in the ribs as described below. Further caudally, four vertebrae are  
97 preserved. One of these is substantially more robust than all others (Fig. 2 F,G. Movie S1),  
98 similar to neural arches preserved in the caudal domain of *Acanthostega*<sup>1</sup>.

### 99 ***Ribs***

100 Specimen NUFV 108 was physically prepared in 2004 and 2005 to expose rostral ribs<sup>13</sup>.  $\mu$ CT  
101 imaging reveals additional ribs preserved beyond those previously identified, making for a total  
102 of 56, including an uninterrupted series of 32 on the left side. Across the series, ribs have a  
103 curved articular head that would have contacted the pleurapophyses of the intercentra. Ribs bear  
104 a flange posteriorly on their proximal portion that varies in its mediolateral span across the  
105 series, and they lack imbricating uncinat processes (Fig. 2 A,B). The rostral-most ribs extend  
106 straight to a tapered, narrow tip. More caudally, at approximately rib number 5, the ribs become  
107 longer and have a gentle ventral curvature. At approximately rib number 20, the ribs shorten in  
108 their mediolateral span and have a broader base, gaining a more triangular shape. Ribs 31 and 32  
109 are markedly distinct in their morphology from others in the series. Rib 31 is broad in dorsal  
110 perspective and has unfinished distal surface that is rounded, while rib 32 shows substantial  
111 ventral curvature as compared more cranial ribs (Fig. 2 A,B). An isolated post-sacral rib is  
112 preserved to the left of the other axial elements (Fig. 1 A,B, Movie S2). Its morphology, narrow,  
113 slightly recurved and posteriorly directed, is similar to the post-sacral ribs of *Acanthostega*<sup>1</sup> and  
114 *Ichthyostega*<sup>2</sup>. No evidence of sternal structures is found.

### 115 ***Reconstruction of the pelvic region***

116 The morphology of the pelvis of *Tiktaalik* was described previously<sup>14</sup>, but, importantly, its  
117 position and relation to the axial column has remained unknown. The right pelvis of specimen  
118 NUFV 108 was preserved adjacent to the axial column, not in articulation<sup>14</sup>. Abrupt transitions in  
119 the morphology of the vertebrae and ribs at position 31 and 32 in *Tiktaalik* denote the trunk-to-  
120 tail transition and likely position of the pelvic girdle. In both *Eusthenopteron* and *Acanthostega*,  
121 transitions in vertebral and rib anatomy at this general position denote the trunk-to-tail transition  
122 and pelvic position. In *Eusthenopteron*, ribs are only present rostral to vertebrae 30, between  
123 vertebrae 30 and 32 the haemal arches enclosed the haemal canal and the become intercentra  
124 fuse bilaterally, and the pelvis is approximately ventral to vertebrae 32<sup>8</sup>. In *Acanthostega*,  
125 vertebrae 31 differs from those immediately rostral in having fused intercentra and bearing a  
126 distinctive and elongate rib with a ventral expansion that would have allowed for attachment to  
127 the girdle, likely *via* soft tissue<sup>1</sup>.

128 New data on the on vertebrae and ribs of *Tiktaalik* allow for assessment of pelvic orientation and  
129 position (Fig. 3, Movie S3). The dorsal extent of the iliac blade of the pelvis would have  
130 approached ribs 31 and 32 of the series. While there is no articular facet on the internal surface  
131 of the girdle for a sacral rib<sup>14</sup>, the proximity inferred from their anatomy, along with a  
132 comparison to other taxa, suggest a soft-tissue linkage. In *Acanthostega*, there is likewise no  
133 distinct articular facet or marked perimeter for the attachment of the sacral rib in the ilium<sup>1</sup>.  
134 Nevertheless, a soft-tissue connection between rib and ilium has been inferred based on rib  
135 morphology and the size and position of the two elements<sup>1</sup>. Similar patterns of connectivity have  
136 been proposed in other early tetrapods, such as *Eryops*<sup>1,18</sup>. A bony articulation between ribs and  
137 pelvis is only definitively present in more crownward tetrapods such as *Whatcheeria*<sup>1,18,19</sup>. The  
138 positioning of the pelvis of *Tiktaalik* suggested by the shape of the pubis and width of the body  
139 (Fig. S1, Supplementary Text) would entail a more posteroventral-facing acetabulum than  
140 previously proposed<sup>14</sup>, more similar to the orientation of the pelvic fins of *Eusthenopteron*<sup>8</sup> than  
141 the laterally positioned limbs of Devonian limbed vertebrates<sup>1,2,20</sup>.

## 142 ***Pelvic fin***

143 Mechanical preparation of specimen NUFV 108 in 2005-2006 exposed parts of the pelvic fin<sup>14</sup>.  
144  $\mu$ CT data reveal new details, including the full extent of the pelvic fin web and additional  
145 endoskeletal elements (Fig. 1, Fig. 4 A). Pelvic fin rays are unbranching and unsegmented.

146 Similar to the pectoral fins of tetrapodomorphs, the pelvic fin rays are more robust on the leading  
147 edge and more gracile on the posterior side<sup>21</sup>. Hemitrichia have accentuated asymmetry. Dorsal  
148 hemitrichia are larger in cross section than ventral hemitrichia, as in the pectoral fin of  
149 *Tiktaalik*<sup>21</sup> (Fig. 4 B). Two new pelvic endoskeletal elements are identified (Fig. 4 A,C). One,  
150 inferred to be a tibia, has a robust proximal articular surface, and its distal margin appears  
151 broken, making it unclear whether a more distal element might have articulated with it. The other  
152 element is small with a posteriorly oriented ventral curving process, a feature not previously  
153 observed in tetrapodomorph pelvic fins<sup>8-10,15,22</sup>.

### 154 **Occipital-vertebral junction**

155 In specimen NUFV 108, the basioccipital-exoccipital complex is preserved apart from the rest of  
156 the skull, medial to the pectoral girdles, and it comprises a bilateral pair of elements (Fig. 1, Fig.  
157 S2 A-D). Examination of  $\mu$ CT data of specimen NUFV 110<sup>23</sup> confirms that the basioccipital-  
158 exoccipital complex is deconsolidated from the rest of the skull in *Tiktaalik*. (Fig. S1 E-I). The  
159 pattern of *Tiktaalik* differs from the general pattern among tetrapodomorphs, where the  
160 basioccipital-exoccipital complex is fused both across the midline and to anterior neurocranial  
161 elements<sup>24,25</sup>. In the tristichopterid *Mandageria fairfaxi*, the basioccipital-exoccipital complex is  
162 also separated from more anterior elements, and this feature has been inferred to allow for  
163 increased notochordal flexion at the occipital-vertebral junction<sup>26</sup>. Deconsolidation of skeletal  
164 elements at the back of the skull in *Tiktaalik*, therefore, provides further evidence for increased  
165 mobility at the head-trunk boundary, which was previously hypothesized based on the absence of  
166 an operculum and extrascapular series<sup>13</sup>.

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### 168 **Discussion**

169 *Tiktaalik* exhibits a unique constellation of primitive and derived characters in the axial skeleton  
170 that suggest it had a locomotor capacity intermediate to currently known finned elpistostegalians  
171 and limbed vertebrates. These data, and the reconstruction they imply (Fig. 5), allow for new  
172 hypotheses on the evolution of axial regionalization and the origin of quadrupedal locomotion in  
173 early tetrapods.

174 The vertebrae of *Tiktaalik* adhere closely to plesiomorphic tetrapodomorph conditions. Most of  
175 the preserved vertebrae are from the trunk, and they are similar to the trunk vertebrae of  
176 *Eusthenopteron* both in degree of differentiation across the series and in overall construction,  
177 except for slight differences in intercentral fusion and the potential lack of pleurocentra<sup>8</sup>. The  
178 number of trunk vertebrae in *Tiktaalik* is similar to other tetrapodomorphs; *Eusthenopteron*,  
179 *Acanthostega*, and *Ichthyostega* are also characterized by approximately 30 pre-sacral  
180 vertebrae<sup>1,2,8</sup>.

181 In contrast to the vertebral column, the ribs of *Tiktaalik* show numerous derived features that are  
182 previously known only from limbed taxa (Fig. 6). As in *Acanthostega*<sup>1</sup> and *Ichthyostega*<sup>2</sup>, the  
183 ribs of *Tiktaalik* extend caudal to the trunk-tail boundary and are regionalized with a sacral  
184 module. This is a departure from plesiomorphic tetrapodomorph pattern, seen in *Eusthenopteron*,  
185 where ribs do not extend caudal to the trunk-tail boundary and those near to the pelvis are not  
186 morphologically differentiated<sup>8</sup>.

187 The rib anatomy of *Eusthenopteron*, coupled with a small ilium positioned ventrally to the  
188 vertebral column, indicate the absence of any linkage between axial column and pelvic fin<sup>8,24</sup>.  
189 The sacral ribs of *Tiktaalik*, on the other hand, would have overlapped the pelvic girdle in lateral  
190 perspective, with ribs lying medial to a large, plate-like ilium. Although there is no evidence of a  
191 bony articulation, the nature of the expansion of both ribs and ilium, the degree of overlap  
192 between the elements, and the unfinished distal margin of one sacral rib, indicates that a soft-  
193 tissue connection was likely in *Tiktaalik*. Such a connection, also proposed to be present in early  
194 limbed forms including *Acanthostega*<sup>1</sup>, likely allowed for a degree of structural support and for a  
195 restricted range of motion between the elements. A soft-tissue linkage between girdle and axial  
196 column would have provided a less robust a connection than direct bony articulations  
197 hypothesized for *Ichthyostega*<sup>4</sup> and observed more clearly in more crownward forms, like  
198 *Whatcheeria*<sup>19</sup>. However, mobility of the pelvic girdle could have allowed for slight changes in  
199 the orientation of the acetabulum during locomotor behaviors. The post-cranial skeleton of  
200 *Tiktaalik*, therefore, reveals that sacro-iliac specializations arose in the ribs and pelvis prior to  
201 modifications to the vertebral column. Subsequent modifications to the axial column observed in  
202 limbed vertebrates include expansion of the dorsal extent of neural arches, either squared as in  
203 *Acanthostega* or rounded as in *Ichthyostega*, and the origin of zygapophyses<sup>1,2</sup>.

204 The presence of sacral ribs, robust pelvis, deep acetabulum, and large pelvic fin in *Tiktaalik*  
205 indicate that the rear appendage was generating greater forces in locomotion than in other finned  
206 elpistostegalians, such as *Panderichthys*. In addition, these features suggest that *Tiktaalik* was  
207 capable of more axial support for the trunk when the pelvic fins were loaded against the substrate  
208 than less crownward elpistostegalians. Despite these apomorphic features, *Tiktaalik* retains  
209 numerous plesiomorphic characteristics in its pelvic anatomy, such a posteriorly facing  
210 acetabulum, left and right pubes unfused along the midline, and lack of an ischium<sup>14</sup>, which  
211 imply that the pelvic fin was not able to retract as extensively as limbed forms such as  
212 *Acanthostega* and *Ichthyostega*. The posterior orientation of the acetabulum of *Tiktaalik* and  
213 concomitant inability to use retraction for limb propulsion suggests that the pelvic fin was unable  
214 to play a significant role in terrestrial walking.

215 With a pelvis and pelvic fin subequal in size to the shoulder girdle and pectoral fin, the overall  
216 proportions of the trunk and paired appendages of *Tiktaalik* hew closer to those of *Acanthostega*<sup>1</sup>  
217 and *Ichthyostega*<sup>2,20</sup> than to *Eusthenopteron*<sup>8,24</sup> and *Panderichthyes*<sup>11,15</sup>. Pelvic and sacral  
218 anatomy implies that *Tiktaalik* represents an intermediate condition in which a large pelvic  
219 appendage was stabilized by the axial skeleton and capable of being used in diverse paddling,  
220 walking, and propping behaviors on aquatic substrates. These functions of the pelvic fin were  
221 antecedents to the terrestrial walking behaviors that were possible in later forms.



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313

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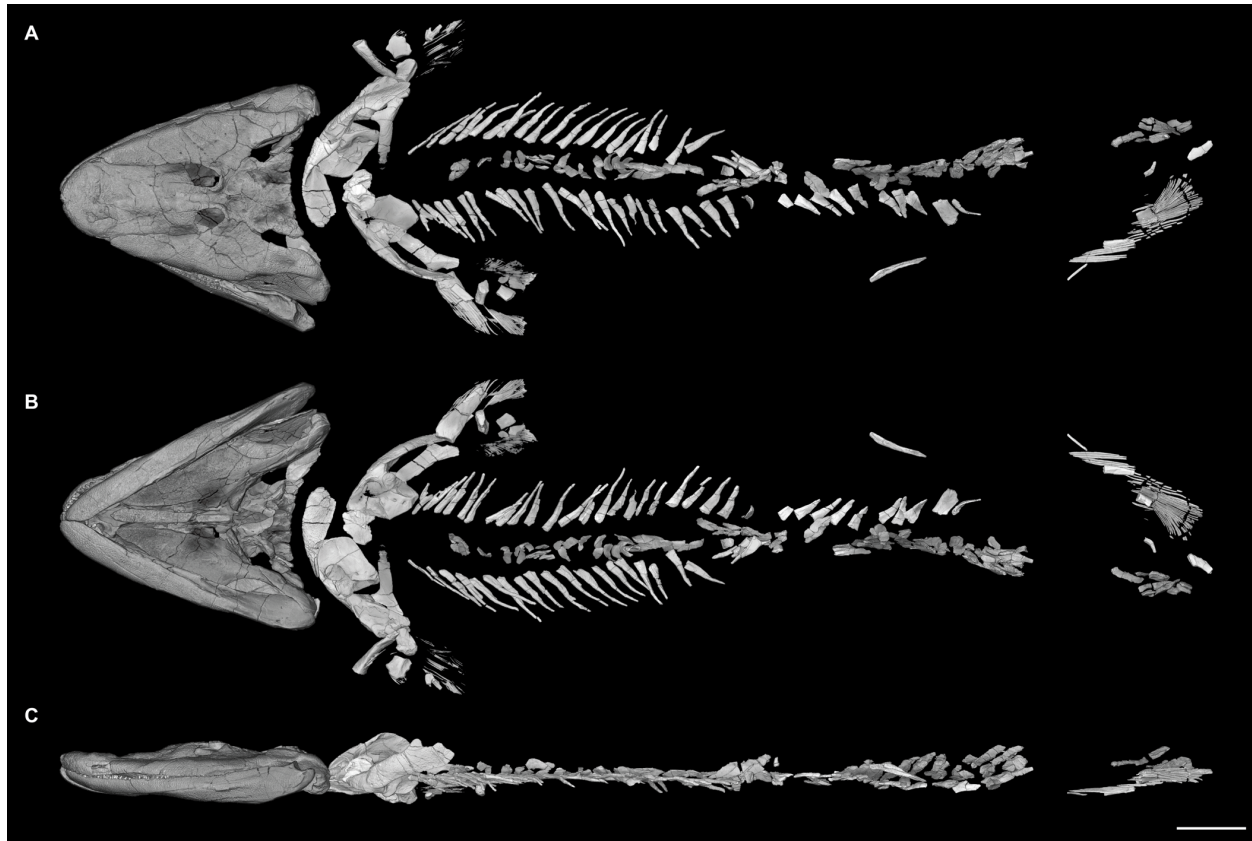
320 **Author contributions:** Field work leaders: NHS, EBD; Funding acquisition: NHS, EBD;  $\mu$ CT  
321 data collection: JBL;  $\mu$ CT data processing: TAS; Visualization: TAS, EJH, IM; Writing –  
322 original draft: TAS, NHS; Writing – review & editing: TAS, JBL, EJH, IM, EBD, NHS

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324 **Competing interests:** Authors declare that they have no competing interests.

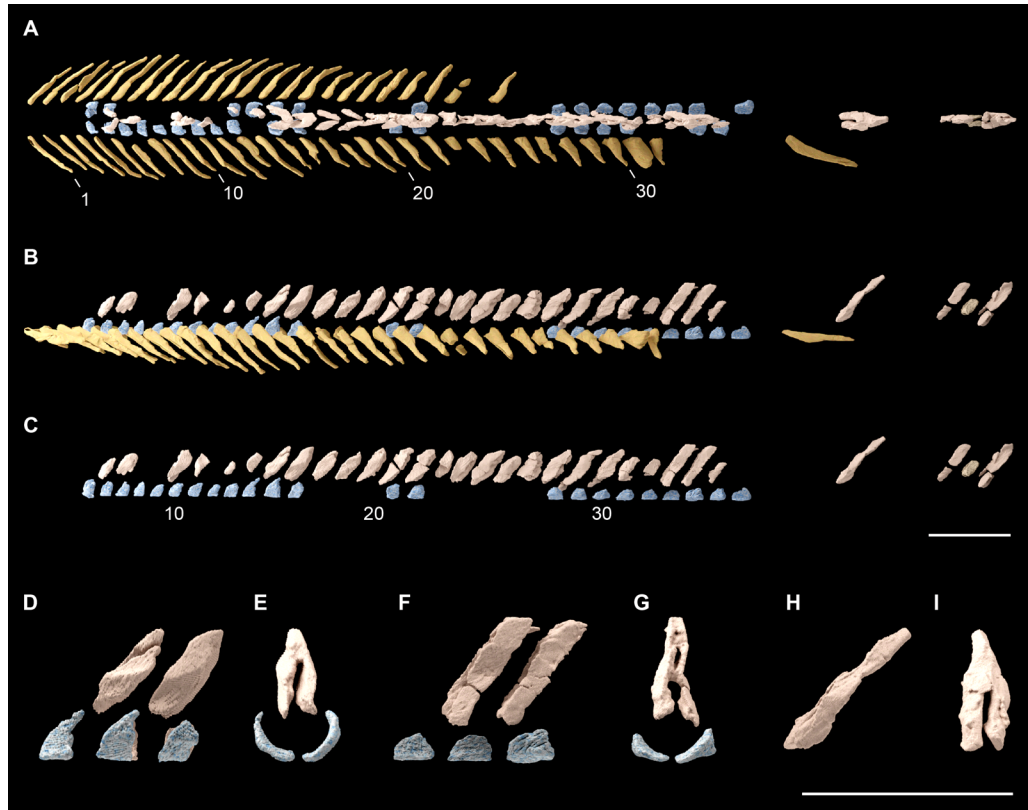
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326 **Data and materials availability:** All data analyzed in the paper will be freely available.  
327 Computed tomography data sets and STL files will be available for download from  
328 MorphoSource prior to publication.



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**Fig. 1. Volumetric rendering of  $\mu$ CT scans of *Tiktaalik roseae*.** Specimen NUFV 108 in (A) dorsal, (B) ventral, and (C) left lateral perspectives.  $\mu$ CT data reveal new detail on the ribs, vertebrae, and pelvic fin. The head, which was mechanically prepared and scanned separately<sup>27</sup>, is positioned slightly anterior to its preserved position. Scale bar, 5 cm.



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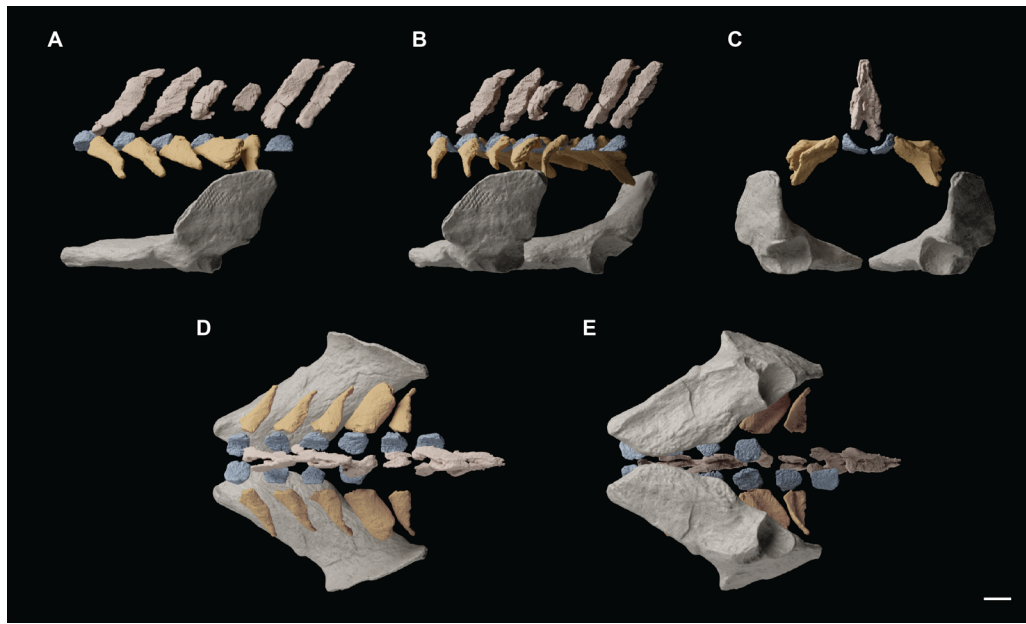
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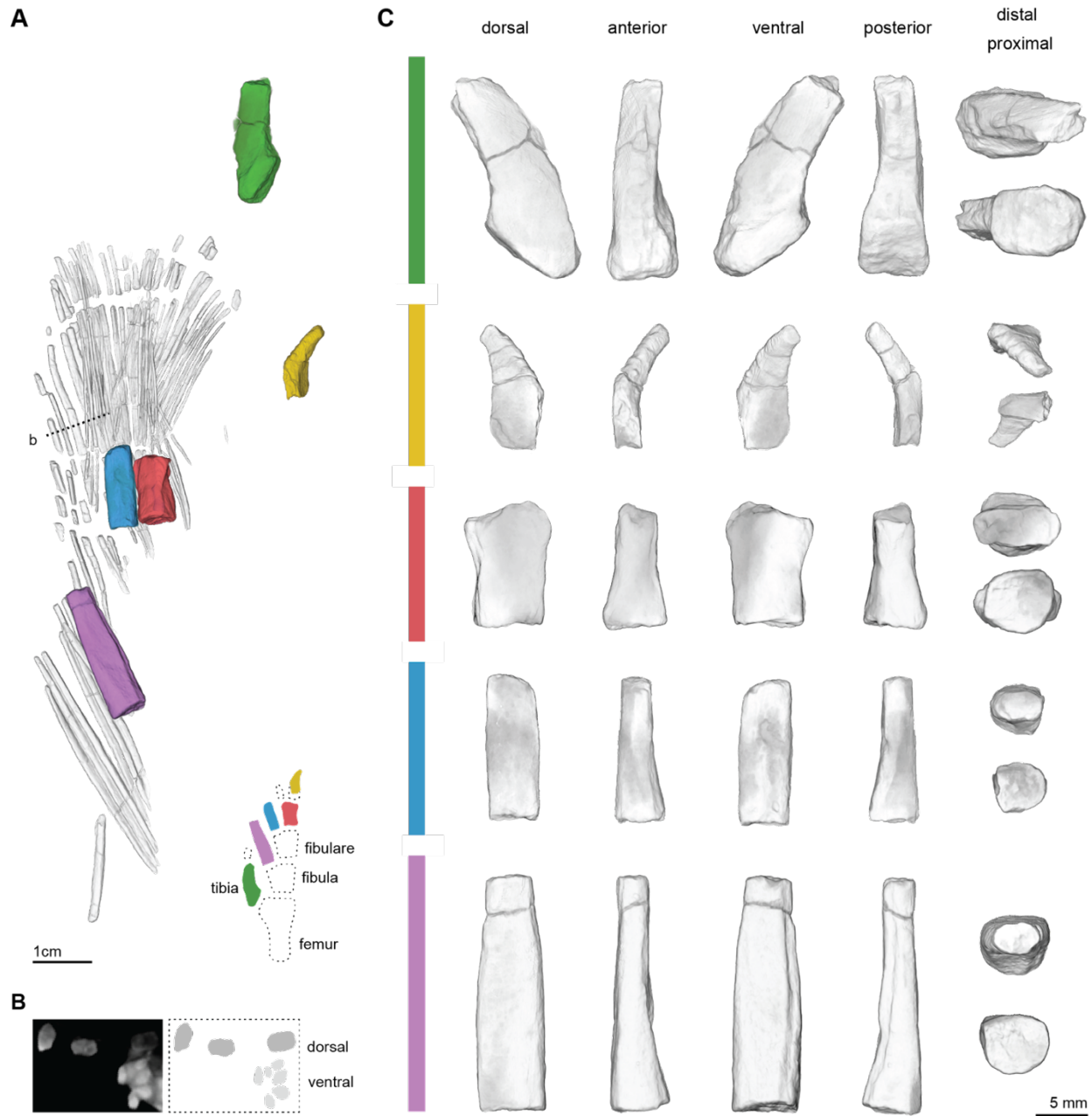
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**Fig. 2. Vertebrae and ribs of *Tiktaalik roseae*.** Vertebrae and ribs in (A) dorsal and (B) lateral perspective. (C) Intercentra and neural arches in lateral perspective. (D,E) Intercentra and neural arches beginning at position 14 in left lateral and anterior perspective (F,G) Intercentra and neural arches beginning at position 32 in left lateral and anterior perspective. (H,I) Neural arch from the caudal region in left lateral and anterior perspective. Ribs are depicted in yellow, neural arches in tan, and intercentra in blue. Scale bars, 5 cm.



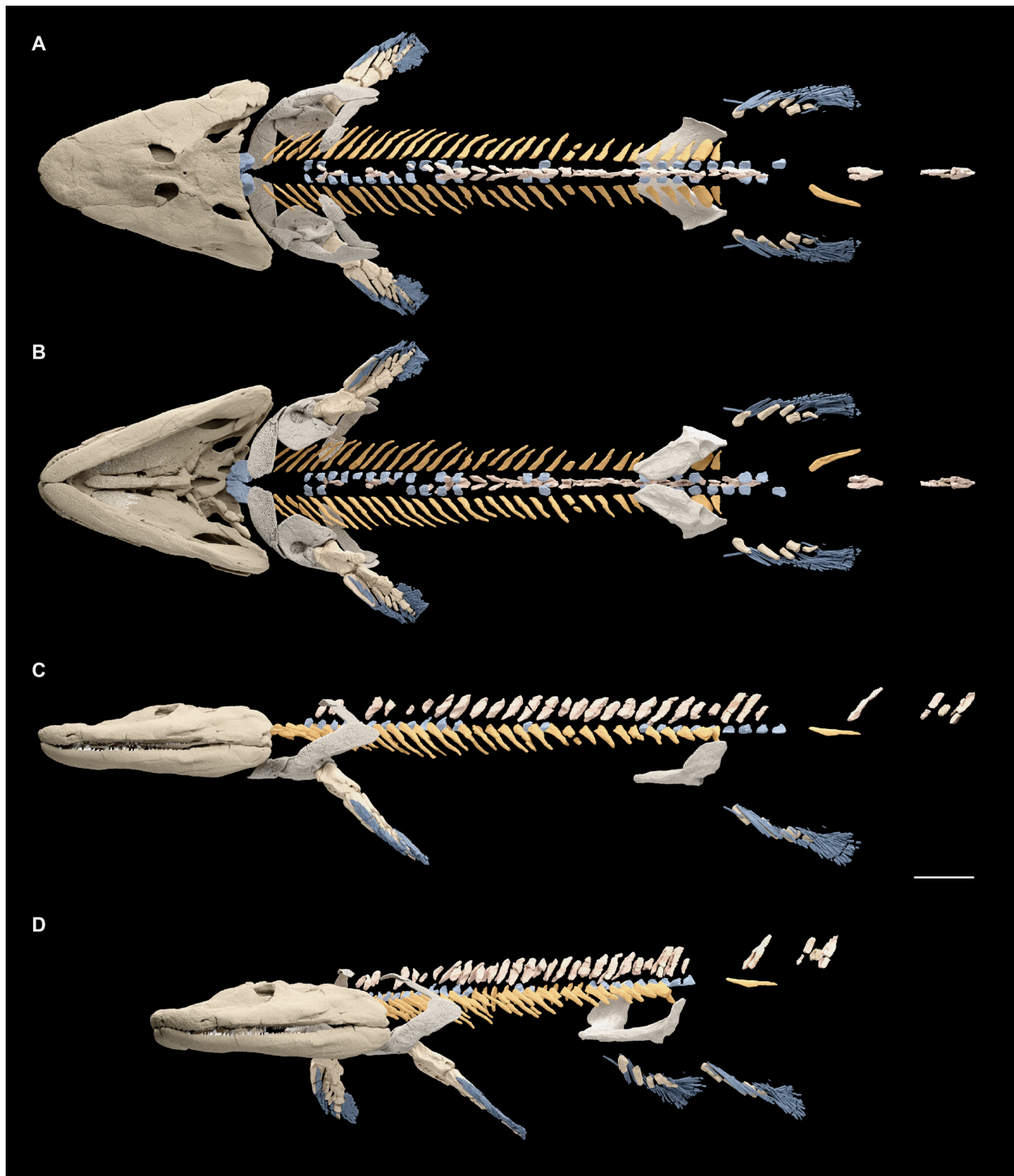
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**Fig. 3. Reconstructed sacral domain and pelvic girdle of *Tiktaalik roseae*.** Reconstruction of the axial column and pelvis in (A) left lateral, (B) posterior-oblique, (C) posterior, (D) dorsal, (E) ventral perspectives. Ribs and pelvic girdle have been mirrored to produce the reconstruction. Ribs 31 and 32 show modified shape as compared to the more anterior elements and are inferred to have supported the pelvic girdle by a soft-tissue connection. Scale bar, 1 cm.



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**Fig. 4. Pelvic fin of *Tiktaalik roseae*.** (A) Volumetric rendering of  $\mu$ CT data of the left pelvic fin of NUFV 108 and a reconstruction of the fin in ventral perspective. (B) Hemitrichia show dorso-ventral asymmetry. The digital cross section, left, and illustration, right, were taken at the position of the dashed line labeled 'b' in panel A. The cross section is oriented orthogonal to the plane of the fin web. (C) Endoskeletal elements of the pelvic fin in various orientations.



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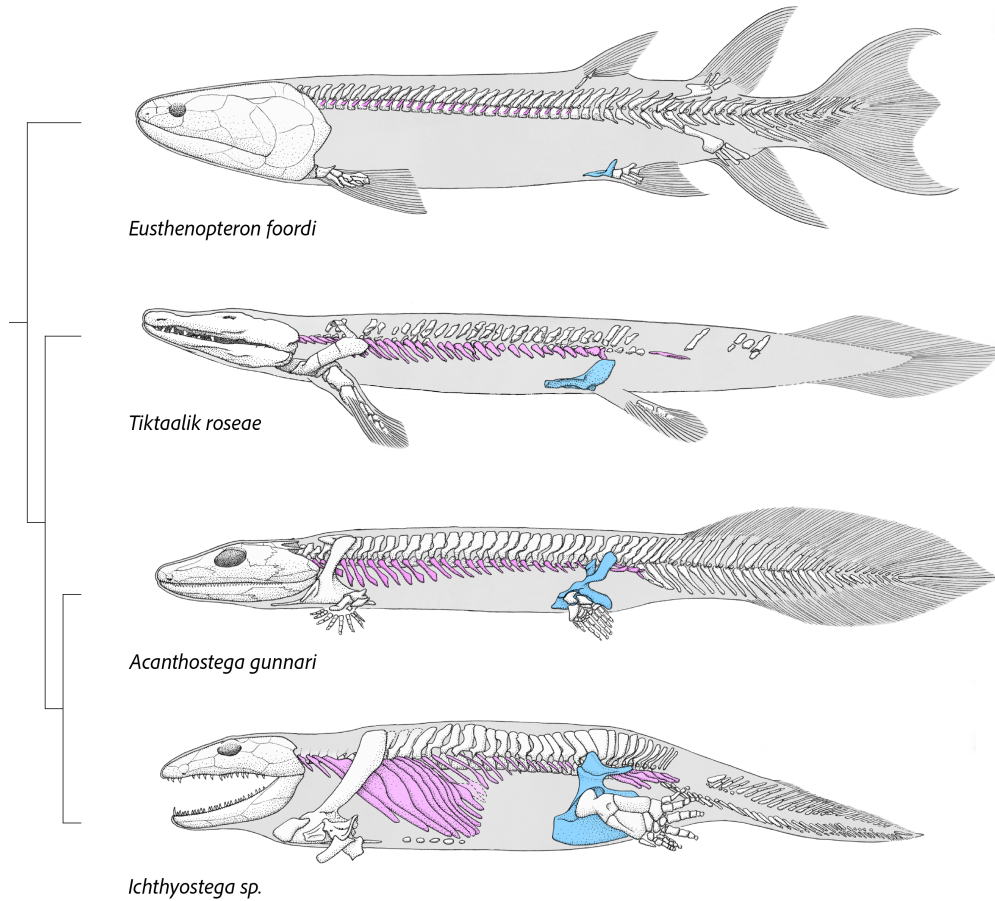
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**Fig. 5. Reconstruction of *Tiktaalik roseae*.** Reconstruction in (A) dorsal, (B) ventral, (C) left lateral, and (D) oblique views. Cranial materials are repositioned according to Lemberg *et al*<sup>23</sup> to account for settling during preservation. Select elements that are preserved from only one side of NUFV 108 (*i.e.*, pre-sacral ribs, pelvic girdle, and fin) are reflected for symmetry. The pectoral fin is from specimen NUFV 110<sup>21</sup> and scaled to the length of the right humerus of NUFV 108. Additional skeletal elements are known for *Tiktaalik*, including branchial skeleton<sup>13</sup> and interclavicle<sup>28</sup>, but have not been rendered here. Scale bar, 5 cm.





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369 **Fig. 6. Reconstructions of Devonian tetrapodomorphs.** The anatomy of *Tiktaalik roseae*  
370 shows that specializations in the axial column for head mobility, body support and pelvic fin  
371 buttressing had evolved in elpistostegalians, prior to the origin of limbs. Illustration of *Tiktaalik*  
372 *roseae* based on specimen NUFV 108. Illustrations of other taxa are based on previously  
373 published descriptions: *Eusthenopteron fordi*<sup>8,24</sup>, *Acanthostega gunnari*<sup>1,2</sup>, *Ichthyostega sp.*<sup>2,20</sup>.  
374 Ribs are depicted in purple. Pelvic girdles are shown in blue.  
375

## Supplementary Materials for

### **Axial regionalization in *Tiktaalik roseae* and the origin of quadrupedal locomotion**

T.A. Stewart, J.B. Lemberg, E.J. Hillan, I. Magallanes, E.B. Daeschler, N.H. Shubin

**This PDF file includes:**

Materials and Methods  
Supplementary Text  
Figs. S1 to S3  
Tables S1  
Captions for Movies S1 to S4

**Other Supplementary Materials for this manuscript include the following:**

Movies S1 to S4

## 396 **Materials and Methods**

397

398 The material of *Tiktaalik roseae* was recovered during paleontological excavations near Bird Fiord on  
399 southern Ellesmere Island in 2004, 2006, 2008, and 2013. All specimens were recovered from a single  
400 locality (NV2K17; N77°09.895' W86°16.157') within the Fram Formation (Frasnian Stage, Late  
401 Devonian). The fossil material is curated in the Nunavut Fossil Vertebrate Collection (NUFV) at the  
402 Canadian Museum of Nature.

403

### 404 Computed tomography scanning

405 CT scans were collected at The University of Chicago's PaleoCT scanning facility with a GE Phoenix  
406 v|tome|x 240 kv/180 kv scanner. The post-cranial skeleton of NUFV 108 is contained in two blocks  
407 (Movie S1). Each of these blocks are too large for single multiscan. Therefore, each block was scanned  
408 each twice: first oriented vertically with the anterior edge down, and then rotated 180 degrees and  
409 scanned again with the posterior edge down. Scanning parameters for these four scans are provided in  
410 Table S1. CT data were reconstructed with Phoenix Datas|x 2 (version 2.3.3) and imported to VGStudio  
411 Max (version 2.2) to be cropped and exported as a tiff stack. For each block, the two multi-scans were  
412 manually stitched together and then manually segmented in Amira (version 20.2) (Thermo Fisher  
413 Scientific).

414

### 415 Surface scanning

416 The pelvis of NUFV 108 was previously physically prepared from the specimen and, therefore, is not  
417 included in the  $\mu$ CT scans. A 3D model of the pelvis was generated by surface scanning a cast of the  
418 pelvis using a FARO Design ScanArm 1.0 at a resolution of 40-75  $\mu$ m.

419

### 420 Images and Animations

421 Volumetric images of the segmented  $\mu$ CT data were generated using Amira (Fig. 1, Fig. 4, Fig. S1). All  
422 other renderings of skeletal elements are surface models, which were generated by exporting  
423 segmentation label fields from Amira as surface files, or directly by surface scanning, and visualized in  
424 Blender (version 3.3.1). Movies were created by first exporting animations as tiff stacks from Amira or  
425 Blender and then using Adobe Premier (version 13.12) to combine and edit the images into movies.

426

427 *Supplementary Text*

428

429 In specimen NUFV 108, individual skeletal elements are three-dimensionally preserved with  
430 minimal deformation, and the specimen has settled during preservation. Previous studies of  
431 *Tiktaalik* have presented reconstructions of the cranial and pectoral fin skeleton based on  $\mu$ CT  
432 data <sup>21,23</sup>. In this study, we describe the axial skeleton of *Tiktaalik* and use  $\mu$ CT data to produce a  
433 three-dimensional model of specimen NUFV 108 that contains nearly all skeletal elements  
434 known for this taxon. Additional elements known from *Tiktaalik* that are not included in the  
435 model include components of the hyoid skeleton and the interclavicle. In generating our model,  
436 numerous decisions were made on how to place the pieces. These decisions are based upon  
437 comparisons between anatomical systems of *Tiktaalik* (e.g., comparing intercentral and neural  
438 arch anatomy, or comparing the pectoral and pelvic girdle), as well as comparisons to other  
439 tetrapodomorphs and extant fishes. The new reconstruction of *Tiktaalik*, thus, represents a  
440 hypothesis based on multiple lines of evidence.

441

442 Reconstruction of the vertebrae

443 Intercentra and neural arches are positioned according to their preserved rostrocaudal order.  
444 Intercentra are assigned to either the left or right sides based curvature of the internal surface and  
445 position of the articular facet. Left and right intercentra that were preserved near to one another  
446 are reconstructed as paired. However, because intercentra are unfused can have shifted during  
447 preservation, it is possible that some elements reconstructed as paired are slightly out of register  
448 from their original position. Intercentra are reconstructed as associated with individual neural  
449 arches. Likewise, it is possible that intercentra could be reconstructed modestly out of register  
450 from their original neural arch. This uncertainty does not impact results presented in the  
451 manuscript.

452 Intercentra are positioned so that they bound the lower portion of the notochord and wrap  
453 dorsally. When both left and right sides are preserved for a vertebra, they are positioned so that  
454 their internal curvature symmetrically fits around a notochord that is circular in cross section.  
455 Intercentra are positioned under the assumption that the notochord is of a uniform cross section  
456 between the head and pelvis, a feature observed in various taxa, including *Eusthenopteron* <sup>8,24</sup>  
457 and *Latimeria* <sup>29</sup>. If only one intercentra was preserved for a vertebra, the element is positioned  
458 so its internal curvature matched elements anterior or posterior it in the series.

459 Neural arches are occasionally broken, and whenever possible the pieces are re-  
460 assembled. Neural arches from vertebrae 5-34 can be associated with ribs or intercentra.  
461 However, four neural arches are preserved more caudally and without clear association to other  
462 axial elements. The anterior-most of these four neural arches is preserved caudal and slightly  
463 ventral to neural arch 32 (Movie S1). Its morphology is significantly more robust than those  
464 immediately anterior (Fig. 2F-I), and it is identified as belonging to the caudal region based of  
465 comparison to *Acanthostega*<sup>1</sup>. Although the neural arch could have been associated with  
466 intercentra 34 or 35, it is depicted in the reconstruction with a gap between it and other elements  
467 to denote uncertainty in position (Fig. 2). The three most-caudal neural arches are preserved in  
468 close association with one another and separated by a substantial gap to other axial elements,  
469 near to the pelvic fin (Fig. 1A,B). These neural arches, too, are depicted in the reconstructed with  
470 gaps between them and other axial elements to denote ambiguity in their position (Fig. 2).

471 To reconstruct the dorsal position of neural arches, we first focused on the most complete  
472 neural arches in the series (e.g., Fig. 2F,G). Despite some lateral compression, these allowed us  
473 to estimate the extent to which the arch would have wrapped around the notochord. When neural  
474 arches were broken, if possible, they are reconstructed so that the apex of their internal curvature  
475 aligns with the apex of other more complete neural arches in the series.

476 To constrain spacing of axial elements in the rostrocaudal direction, we considered the  
477 preserved distance between ribs 1 and 32 in NUFV 108 to approximate the distance between ribs  
478 1 and 32 in life. Across this distance, ribs and vertebrae are placed so that gaps between the  
479 vertebrae were uniform, except when their position was uncertain (see discussion above of the  
480 caudal-most 4 vertebrae and discussion below of the sacral rib). Vertebrae 32-36 are spaced at  
481 distances similar to those of positions 1-32.

482 In the reconstruction of *Tiktaalik*, intercentra are positioned slightly anterior to their  
483 corresponding neural arch. This positioning is based on several features. First, the positioning of  
484 intercentra reveals the size of the notochord, and comparison of intercentral and neural arch  
485 morphologies suggest that they are unlikely to have been aligned strictly dorsally, because this  
486 would have produced a lateral overlap of the elements. Second, pleurocentra were not identified  
487 for specimen NUFV 108. If large pleurocentra had been observed in the specimen, then the  
488 vertebrae are likely to have been organized such that neural arches were positioned dorsal to  
489 their corresponding intercentra, as in *Osteolepis*<sup>9</sup>. Therefore, the absence of pleurocentra

490 indicates they were either small or fused to the intercentra; both conditions predict that neural  
491 arches and intercentra were not vertically aligned, but slightly out of register<sup>3,8</sup>.

492 The neural arches of *Tiktaalik* lack zygapophyses. This suggests space between adjacent  
493 neural arches. Therefore, they are situated with angles of inclination that maintain a slight gap  
494 between adjacent elements. The caudal four neural arches are positioned with similar angles of  
495 inclination as those in the trunk series.

496

#### 497 Reconstruction of the ribs

498 The anterior-most rib on the left side is broken in two pieces, which were preserved in contact  
499 with one another with a sharp angle between them (Fig. 1). These pieces are placed end-to-end to  
500 reconstruct the original element (Fig. 2). Other ribs that are broken have pieces preserved in  
501 close proximity with one another, and they are approximately aligned (e.g., rib 23 on the right  
502 side). In the reconstruction, the pieces of these other broken ribs are kept in their preserved  
503 positions and have not been moved closer to one together in the reconstruction. This presentation  
504 was done to preserve information on which features are broken and not to imply that any gaps in  
505 individual ribs represent their original length and missing portions of the rib.

506 Two ribs on the left side (ribs seven and twelve) and one on the right side (rib six) were  
507 displaced during preservation such that the distal portion of the rib was posteriorly oriented and  
508 ventral to the rib that followed. Additionally, four ribs on the right side (ribs 20-23) are preserved  
509 such their articular surfaces point posteriorly. In each of these cases, the individual ribs were  
510 rotated and repositioned preserving the order of their proximal articular surfaces.

511 One rib is preserved to the left of the rest of the axial series, and it is identified as a post-  
512 sacral rib. It is possible that it might have articulated upon intercentra 33-36, as approximately 5  
513 post-sacral ribs are preserved in *Acanthostega*<sup>1</sup> and *Ichthyostega*<sup>2,20</sup>. However, the rib is  
514 depicted in the reconstruction with a gap between it and other axial elements to denote ambiguity  
515 in position.

516 Ribs were positioned relative to the vertebral column based on curvature of the proximal  
517 articular surface. In many ribs, this portion is broken or incomplete. Therefore, across the series,  
518 ribs are placed by first reconstructing the positions of those ribs with complete articular heads.  
519 These ribs were placed so that their heads aligned with the curvature of the posterior margin of  
520 the intercentra, which bear an articular facet. Ribs with damaged heads were then positioned to  
521 maximize similarity in their orientation to those with complete heads.

522

523 Reconstruction of the pelvis

524 Rostrocaudal positioning of the pelvis of *Tiktaalik* is based upon transitions in rib and neural  
525 arch anatomy. Specifically, the girdle is placed so that the dorsal extent of the ilium is  
526 rostrocaudally aligned with the sacral ribs (ribs 31 and 32). This positioning is similar to what  
527 has is proposed for the pelvic girdles of *Acanthostega* and *Ichthyostega*, which each have  
528 approximately 30 pre-sacral vertebrae <sup>1,2</sup>.

529 Dorsovenral positioning of the pelvic girdle of *Tiktaalik* is based on comparisons to  
530 other tetrapodomorphs. Uniformly, tetrapodomorphs are reconstructed with the ventral portion of  
531 the pelvic girdle approximately in line with the ventral portion of the pectoral girdle (*e.g.*,  
532 *Eusthenopteron* <sup>8</sup>, *Acanthostega* <sup>1</sup>, *Ichthyostega* <sup>2</sup>). In *Tiktaalik*, thus, the pelvic girdle is placed  
533 with a position that comports with the body thickness observed in the articulated pectoral region.

534 To reconstruct the medio-lateral splay of the pelvic girdle of *Tiktaalik*, first the  
535 anteromedial portion is positioned near to the midline, as in *Eusthenopteron* <sup>8</sup>. Next, the girdle  
536 was positioned to produce a taper in the body outline when viewed from the dorsal perspective.  
537 Specimen MHNM 06-2067 of *Elpistostege* shows approximately 30% reduction in the width of  
538 the trunk between the pectoral and pelvic fins <sup>30</sup>. The pelvic girdle of *Tiktaalik* is reconstructed  
539 similarly, resulting in a narrow distance between the ilium and sacral ribs (Fig. S1 A-E). This  
540 reconstruction predicts a more posterior orientation of the acetabulum than previously  
541 hypothesized <sup>14</sup>, one approximately similar to *Eusthenopteron* <sup>8</sup>. We regard this hypothesis of  
542 pelvic positioning as more likely than one where the dorsal extent of the ilium is parallel to the  
543 axial column (Fig. S1 F-I). Such a wide splay would result in an unusually ovate shape of the  
544 trunk in cross section at the position of the pelvis (Fig. S1 H), which is not known among  
545 tetrapodomorphs. Additionally, if a lateral orientation is constrained, but the angle between left  
546 and right halves is increased to produce a more rounded cross-section, this increases the height of  
547 the girdle in lateral perspective and yields a reconstruction where body thickness is greater at the  
548 pelvis than the pectoral girdle (Fig. S1 H). As noted above, such an increase in body thickness is  
549 not seen in other closely related taxa and regarded as unlikely.

550 Thus, positioning of the pelvis is constrained by both features of other anatomical  
551 systems (*i.e.*, vertebrae, ribs, and pectoral girdle) and by comparisons to other tetrapodomorphs.  
552 Although there is uncertainty in some features of the reconstruction, alternative hypotheses of  
553 pelvic girdle positioning for *Tiktaalik* robustly predict that the dorsal extent of the ilium

554 approached the sacral ribs and that they overlapped in lateral perspective. Further, alternative  
555 predictions also recover a pelvic fin of *Tiktaalik* that is more posteriorly oriented than in  
556 *Acanthostega* and *Ichthyostega*<sup>1,2</sup>.

557

#### 558 Reconstruction of the pelvic fin

559 A line drawing of the pelvic fin is presented in Fig. 4 A that shows the estimated positions of the  
560 preserved endoskeleton elements as well as estimates of the geometry of missing elements.

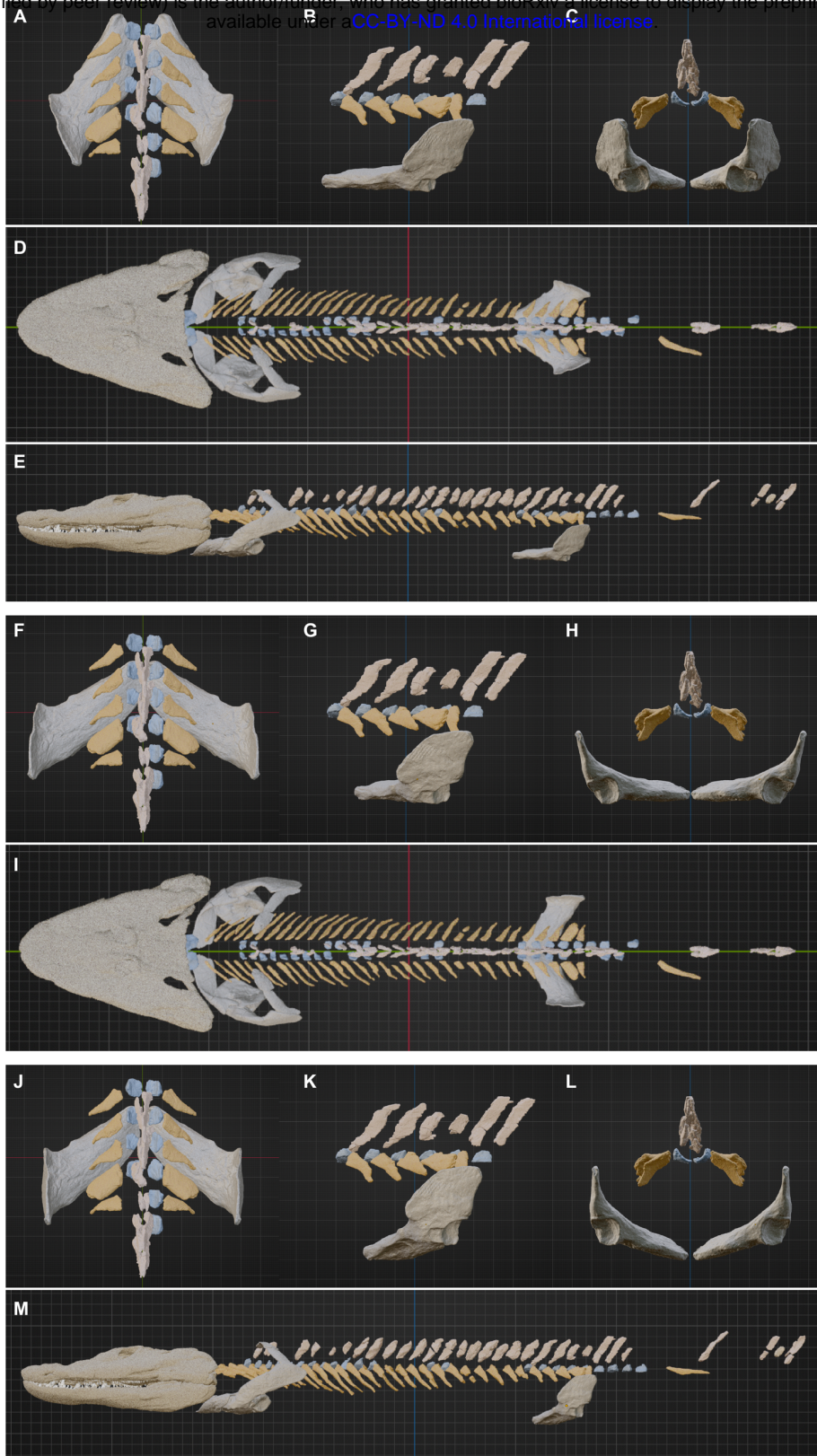
561 Along the proximodistal axis, fins generally taper dorsoventrally. Accordingly, proximal skeletal  
562 elements have articular surfaces that are deeper in the dorsoventral direction than those more  
563 distally positioned. As previously noted, element shown in purple in Fig. 4 has a similar  
564 morphology to the intermedium of the pectoral fin of *Tiktaalik*<sup>14</sup>; it is, thus, reconstructed as  
565 articulating with the fibula. This positioning contributed to the identification of the tibia. The  
566 element identified as the tibia has an articular surface deeper dorsoventrally than any other  
567 preserved pelvic endoskeletal elements and, therefore, would likely have been more proximally  
568 positioned than the element shown in purple. The general pattern of tetrapodomorph pelvic fins  
569 is such that one would predict only three possible more proximal elements: the femur, fibula, and  
570 tibia. The geometry of this most robust element is inconsistent with either a femur or fibula, both  
571 of which likely would have had two distal articular facets, and it is therefore identified as the  
572 tibia.

573 In the drawing, the tibia is illustrated with a dashed component distal to it. The distal  
574 geometry of the tibia is rough and uneven as compared to the distal surfaces of other pelvic  
575 elements, like the intermedium, third mesomere, and third anterior radial. Therefore, this texture  
576 is taken to indicate that the distal portion of the tibia might have broken off or was poorly  
577 ossified. It is possible that a small element articulated distally with the tibia. We regard this  
578 condition as unlikely, because neither *Eusthenopteron*<sup>8</sup> nor *Panderichthys*<sup>15</sup> have pelvic fins  
579 showing an element articulating distally with the tibia.

580 Several endoskeletal elements of the pelvic fin are not preserved. Their approximate  
581 geometries are estimated in the illustration. Mesomeres are typically not longer proximodistally  
582 than those more proximal to them. Therefore, we estimated the relative lengths of the fibulare,  
583 fibula based on the third mesomere (shown in red in Fig. 3). The approximate geometry of the  
584 femur is based on the assumptions that it would be at least as long as the tibia and distally wide  
585 enough to accommodate the tibia and fibula.



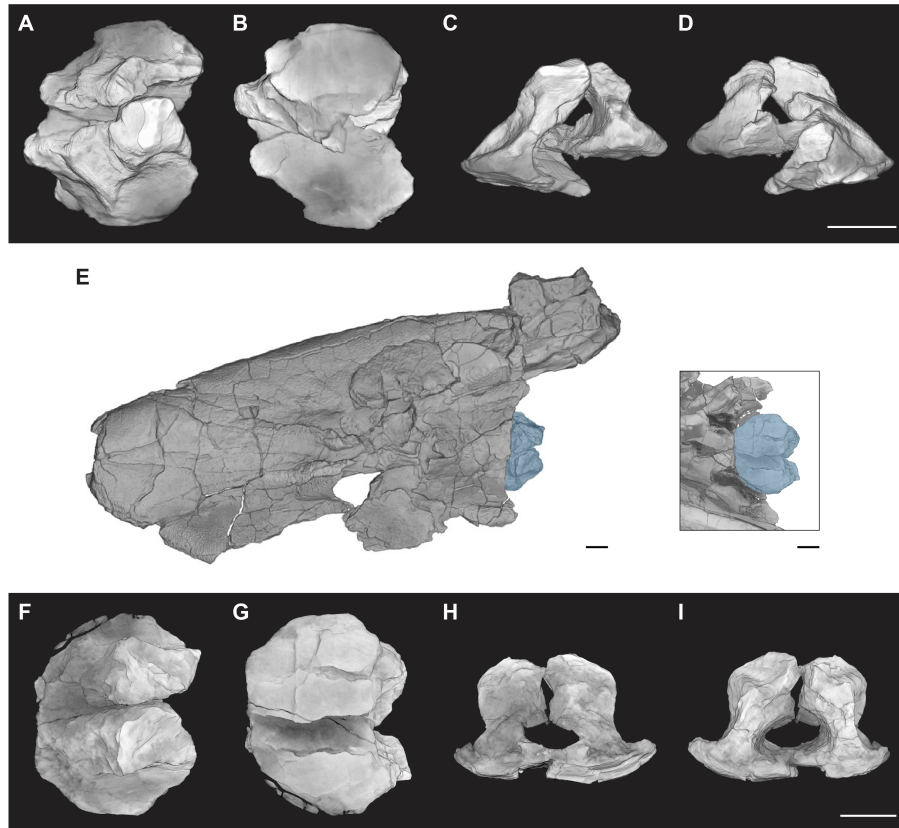
586                   In the pectoral fin, fin rays overlap with the radius. The tibia, the homologous element in  
587 the pelvic fin, is therefore expected to similarly have been covered by lepidotrichia. Accordingly,  
588 it is positioned in the 3D reconstruction so that dorsal hemitrichia would have reached  
589 approximately to the base of the femur. Individual fin rays within the fin web are not  
590 repositioned. The pelvic fin is placed relative to the girdle such that a femur, if present, would be  
591 extending straight from the acetabulum.



**Fig. S1.**

**Alternative reconstructions of pelvic girdle of *Tiktaalik roseae*.** Alternative hypotheses for the positioning of the pelvic girdle were considered when building the reconstruction, as reviewed in the Supplementary Discussion. Panels A-E show the reconstruction of the pelvic girdle presented in the main manuscript. Panels F-I compare that condition with an alternative positioning, where the dorsal extent of the ilium is parallel to the rostro-caudal axis and the ventral aspect of the

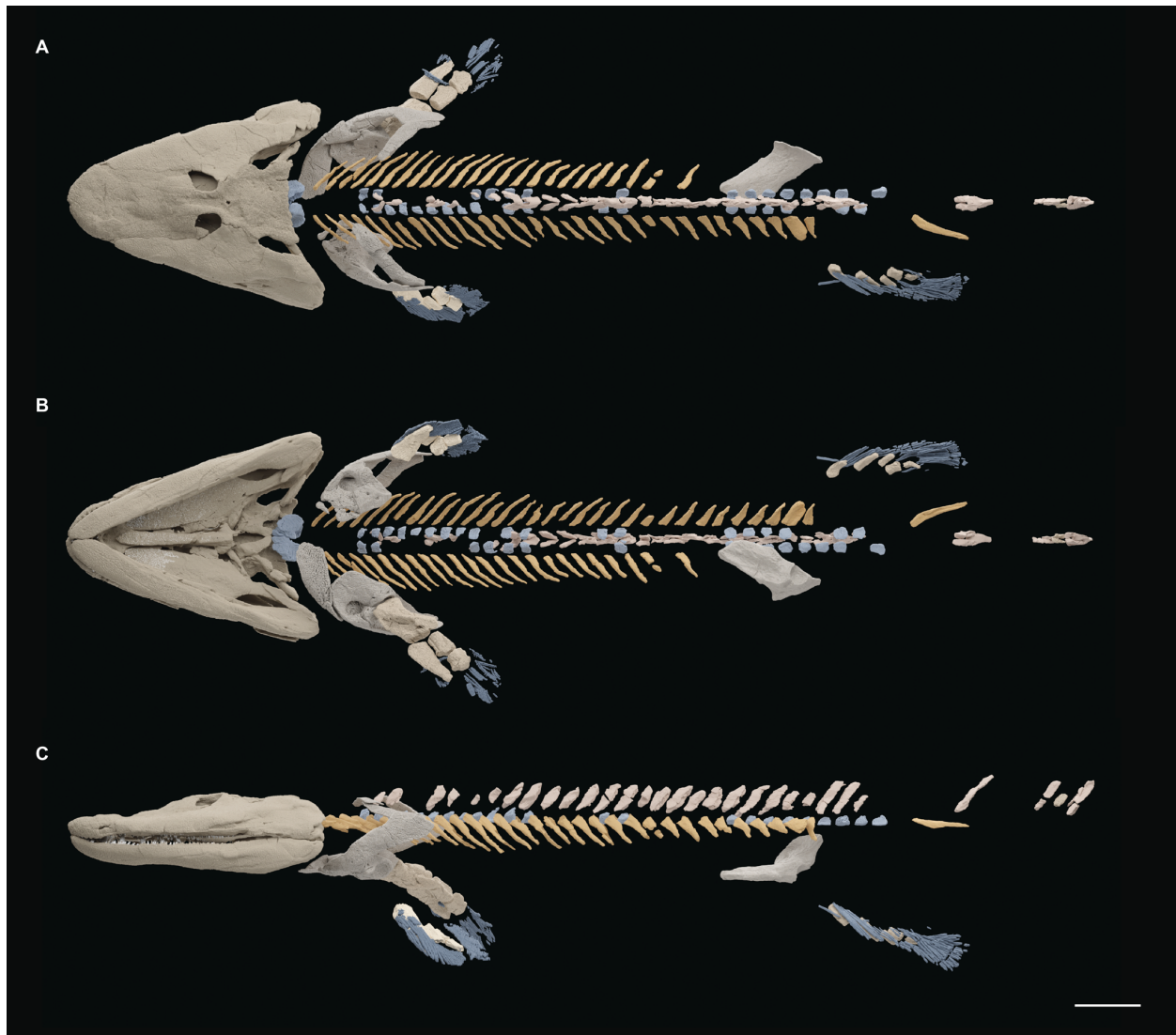
623 pelvic girdle is aligned with the ventral aspect of the pectoral girdle. This position, with a broad  
624 body in the pelvic region, corresponds to previous reconstruction of the pelvic girdle<sup>14</sup>. Panels J-  
625 M show a third reconstruction, where the left and right halves of the pelvis are rotated.  
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**Fig. S2.**

**Basioccipital-exoccipital complex of *Tiktaalik roseae*.** The basioccipital-exoccipital complex of *Tiktaalik* is preserved in specimens NUFV 108 and NUFV 110 as paired elements that are unfused to the rest of the braincase. In NUFV 108, the elements are preserved medial to the pectoral girdle, as depicted in Fig. 1. The basioccipital-exoccipital elements of NUFV 108 shown in preserved positions from (A) dorsal, (B) ventral, (C) anterior, and (D) posterior perspectives. (E) In NUFV 110, the basioccipital-exoccipital complex is still contacting the rest of the skull. The basioccipital-exoccipital complex of NUFV 110 from (F) dorsal, (G) ventral, (H) anterior, and (I) posterior perspectives. Scale bars, 1 cm.



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**Fig. S3.**

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**Specimen NUFV 108 with elements repositioned.** Rendering of all skeletal elements of NUFV 108 that have been  $\mu$ CT scanned are shown here in their reconstructed positions. These images differ from the reconstruction in Fig. 5, which shows several elements duplicated for left-right symmetry and coupled with the more complete pectoral fin of another specimen. Scale bar, 5 cm.

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**Table S1.**

scan ID	Voltage (kV)	Current ( $\mu$ A)	Voxel Size ( $\mu$ m)	Filter (mm)	Scan Duration
pectoral block anterior	100	570	122.441	0.24 Cu	1hr44min
pectoral block posterior	100	570	88.038	0.24 Cu	5hr06min
pelvic block anterior	110	450	73.23	0.24 Cu	6hr48min
pelvic block posterior	110	450	73.23	0.24 Cu	6hr48min

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651

Parameters for  $\mu$ CT scanning of specimen NUFV108

- 652 **Movie S1.**  
653 Volumetric rendering of the two blocks containing the post-cranial skeleton of NUFV 108  
654 including matrix  
655
- 656 **Movie S2.**  
657 Volumetric rendering of NUFV 108 with all segmented elements in their preserved position  
658
- 659 **Movie S3.**  
660 Rotation of the reconstructed sacral domain of *Tiktaalik roseae*  
661
- 662 **Movie S4.**  
663 Rotation of the reconstruction of *Tiktaalik roseae*